multidomain Transferrin (Tf) complex. This state deconvolution from SAXS is achieved via a Monte Carlo (MC) algorithm seeking the optimization of two-state theoretical scattering profiles against a single SAXS profile of Tf. First, CG simulations are used to characterize the Tf structural flexibility and a recently developed Fast-SAXS method is used to compute SAXS profiles of two Tf states. Second, the details of MC simulations for SAXS analysis are illustrated and the choices of scoring functions are discussed. Finally, we examine the extent to which SAXS data can be used to derive the Tf state population (including averages and uncertainties). Our results demonstrate that the use of the Rg-determining region of the SAXS data (q is up to 0.025/Å; referred to as low-q) fails to determine the Tf state population averages. Instead, the SAXS data beyond the low-q region (q is up to 0.15/Å) is required for Tf population reconstruction. Furthermore, the use of a higher-q region of SAXS data (q is up to 0.3/Å) is shown to improve the population precision by reducing the uncertainties, although with no effect on the accuracy of the averages. Thus, the development of MC-based SAXS analysis and its application to the multidomain Tf complex provide the theoretical basis of using SAXS data for studying macromolecular assemblies.

1696-Pos Board B606

Automated, Simultaneous Small- and Wide-Angle X-ray Scattering from Protein Solutions

Lin Yang, John Trunk, John Skinner, Marc Allaire.

We describe the instrumentation for protein solution X-ray scattering at beamline X9 of the National Synchrotron Light Source. By utilizing two detectors, we collect scattering data within a continuous q-range of ~0.005-2.0Å^{-1}. The scattering data from the two detectors are merged and processed using a set of Python scripts. The vacuum-compatible sample cell features flat windows that produce minimal scattering background. The concentration of the protein sample is quantified by measuring the UV-Vis absorption spectrum near the X-ray beam spot. Samples stored in 0.6ml micro-centrifuge tubes can be measured in groups of 15 automatically. The instrument can also be used together with a size exclusion chromatography to measure freshly purified protein samples.

1697-Pos Board B607

Accelerated Macromolecular Solution Shape Recovery from Small Angle Scattering Data

Haiguang Liu, Petrus H. Zwart.

Low-resolution macromolecular shapes as obtained from a Small Angle X-ray Scattering (SAXS) analyses often can provide low-resolution macromolecular shapes of macromolecules and their complexes and important insights in biological function. We present a novel and computationally efficient method for shape reconstruction from SAXS data, operating at accelerated speeds. The program can find high quality molecular shapes in less than two minutes, thus providing real-time feedback during SAXS experiments. The methods are implemented in the program sastbx.shapeup as part of an open source software package, 'the small angle scattering toolbox (sastbx)'.

The molecular shapes are represented using orthogonal 3D polynomials, the zernike polynomials. The shape databases can be customized to generate more relevant results. The results are validated from systematical tests with simulated and experimental data. Automated real time feedback to experimentalists can enhance experiment design and throughput.

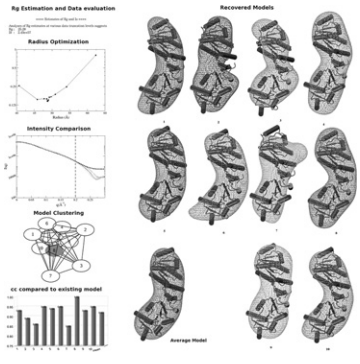

Figure 1. Summary of sastbx.shapeup results, highlighting model recovery based on simulated data of PISA model 2P6N. In 81 seconds, sastbx.shape estimates the radius of gyration, and simultaneously optimizes the radius while searching for the best model. Note that proposed model #8 is constructed from model 2P6N.

1698-Pos Board B608

Biomolecular Mechanisms of Microbial Mercury Resistance in the Environment

Alexander Johs, Jerry M. Parks, Ian Harwood, Hao-Bo Guo, Jeremy C. Smith, Susan M. Miller, Anne O. Summers, Liyuan Liang.

Microbes have naturally evolved to deal with heavy metal toxicity resulting in elaborate heavy metal resistance mechanisms. Bacterial mercury resistance is mediated by the mer operon. It encodes specific genes that facilitate removal of toxic mercury species from the cell. Here, we apply experimental biophysics and high performance computer simulation to investigate molecular mechanisms of bacterial resistance to mercury.

Expression of mer genes is mediated by MerR, a metal-responsive transcriptional regulator. In vitro studies have shown that MerR forms a non-transcribing pre-initiation complex with RNA polymerase and the promoter DNA. We have used small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations to describe changes in the conformation of MerR in response to Hg (II). Our results show that Hg(II) triggers a reconfiguration of the MerR dimer, from a compact state to a conformation revealing two distinct domains. Such a dramatic change in the MerR structure may be the driving force behind a reorientation of DNA recognition sites and ultimately result in initiation of transcription. MD also revealed large amplitude motions in the two DNA-binding domains on a nanosecond timescale.

Reduction of Hg(II) to Hg(0) is catalyzed by mercuric reductase, MerA. All MerA proteins have a homodimeric catalytic core and many have an N-terminal metallochaperone-like domain, NmerA, which acquires Hg(II) and transfers it to an active site in the core homodimer for reduction. We have applied SAXS and MD simulations to explore the structure and dynamics of full length MerA and the docking site of NmerA with core. Our data show that the two N-terminal domains in dimeric MerA sample a large number of conformations and that the transfer of Hg(II) between NmerA and the catalytic core is mediated by transient docking of NmerA to the catalytic core near the protomer interface.

Vibrational Spectroscopy

1699-Pos Board B609

Chiral Sum Frequency Generation Spectroscopy Provides a Set of Optical Vibrational Markers to Distinguish Protein Secondary Structures at Interfaces

Elsa C.Y. Yan, Li Fu.

Quantitative characterization of protein secondary structures at surfaces is important in biological and biomedical sciences. However, probing secondary structures and their changes at surfaces remains technically challenging. We applied chiral sum frequency generation (SFG) spectroscopy to analyze secondary structures at interfaces. We identified a set of vibrational optical markers to distinguish random coils, alpha-helices, and beta-sheets. We found that a monolayer of alpha-helical proteins, including rhodopsin and alamethicin, yields chiral vibrational signals at 3280-3350 cm^{-1} corresponding to the N-H stretch of the peptide backbones, which are not detected from random coils and beta-sheets. We also observed the chiral amide I stretch from amyloid aggregates formed by islet amyloid polypeptide (IAPP) at 1620^{-1} cm, a vibrational signature for parallel beta-sheet distinct from that for anti-parallel beta-sheet at 1630 cm^{-1}. We combined these results with our observations that random coils are silent in chiral SFG spectra and derived a set of markers that allow identification of secondary structures. We further used these markers to investigate the misfolding of IAPP at the lipid/water interface and observed the conversion of IAPP from random-coil to alpha-helical structures followed by parallel beta-sheets in the time scale of hours upon interaction with lipid membranes. Our results demonstrate that chiral SFG spectroscopy allows not only characterization of static structures, but also investigations of kinetics. Because chiral SFG spectroscopy is insensitive to achiral solvent, it is relatively background-free. As a second-order optical method, it provides vibrational signals in the visible region for easy detection. These characteristics combined with intrinsic surface selectivity make chiral SFG spectroscopy complementary to other vibrational methods in studies of protein conformational changes and ultrafast vibrational dynamics for solving fundamental and engineering problems at the molecular level.

1700-Pos Board B610

Low-Frequency Raman Spectroscopy of Trialanine

Rachel M. Stephenson, Angela R. Hight Walker.

The effect of sample conditions on the structural conformation of trialanine has been investigated with visible Raman spectroscopy. Trialanine is used here as a simple protein-mimetic system in order to more easily isolate the backbone amide vibrational modes, low-frequency tortional modes and modes from hydrogen bonding. Crystalline trialanine is known to exist with both parallel (p-Ala3) and antiparallel (ap-Ala3) β-sheet crystal structures, depending on the solvent composition during crystallization. The ap-sheet form of trialanine co-crystallizes with water, which is easily removed under vacuum, offering a further opportunity to examine the effect of solvation on the vibrational spectra, especially when also compared with trialanine in solution. By collecting Raman spectra in different sample phases, and at different concentrations, pH and temperatures, the vibrational modes most sensitive to the secondary structure can be identified. The collected data will be compared to